Lasers in Surgery and Medicine 24:276–284 (1999)

Photodynamic Activity of Lutetium-Texaphyrin in a Mouse Tumor System

Marie J. Hammer-Wilson, MS, Marjan Ghahramanlou, MA, and Michael W. Berns, PhD*

Beckman Laser Institute and Medical Clinic, University of California, Irvine, California 92697

Background and Objective: **New photosensitizers proposed for photodynamic therapy (PDT) treatment of tumors need to be evaluated in animal models to determine the parameters needed for treatment. They also need to be compared with existing photosensitizers for efficacy. We examined the PDT response to lutetium-texaphyrin (PCI-0123) in a mouse mammary adenocarcinoma model and compared it with the PDT response seen when using Photofrin®.**

Study Design/Materials and Methods: **DBA/2 mice with SMT-F tumors were used to explore PCI-0123 toxicity, laser light dose, and drug dose effects on PDT response and to determine the most effective time for light application. The PDT response of PCI-0123-treated tumors was compared with that of Photofrin®-treated tumors.**

Results: **Treatment of tumors with 150 J/cm² of 740 nm laser light 5–6 hr after PCI-0123 administration (40 mg/kg) resulted in a 100% response rate and a 55% cure rate. Tumors treated with 150 J/cm² of 630 nm laser light 24 hr after Photofrin® administration (10 mg/kg) resulted in a 67% response rate and a 16% cure rate.**

Conclusion: **PCI-0123 was found to be a more effective photosensitizer than Photofrin®. Lasers Surg. Med. 24:276–284, 1999.**

Key words: lasers; photodynamic therapy; Photofrin®; photosensitizers; SMT-F tumor

INTRODUCTION

Photodynamic therapy (PDT) is based on the principle that certain compounds can be activated by specific wavelengths of light to produce cytotoxic effects in the tissue where they are located [1]. Successful PDT of cancer depends on the relative selective localization and/or retention of a photosensitive compound within the malignant tissue, such that it can produce light-mediated damage in the tumor without causing significant damage to the surrounding normal tissue [2,3]. Photofrin® is the only currently approved photosensitizer for PDT in humans in the United States. However, clinical trials using other photosensitizers for the PDT treatment of a variety of tumors have been conducted with promising overall results [4].

With the exception of 5-amino-levulinic acid (ALA), the most successful photosensitizers are porphyrins (Photofrin®), porphyrin derivatives (chlorins, purpurins, benzoporphyrin), or compounds designed to be like porphyrin in structure

Contract grant sponsor: NIH; Contract grant numbers: 2R01CA32248 and RR01192; Contract grant sponsor: DOE; Contract grant number: DE-FG03-91ER61227.

*Correspondence to: Dr. Michael W. Berns, Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, CA 92697-1475.

Accepted 20 November 1998

(phthalocyanins). ALA is effective because it is a precursor of the photosensitizer protoporphyrin IX [1,4]. When these compounds absorb sufficient light at the appropriate wavelengths, photochemical reactions are initiated, which in the presence of oxygen result in the production of both singlet oxygen and superoxide anions [5]. When located inside a cell, these highly reactive oxygen products can cause further oxidative reactions within the local environment, resulting in various types of intracellular damage [1]. All of these compounds have absorption peaks between 630 nm (Photofrin®, ALA) and 690 nm (benzoporphyrin derivative mono-acid ring A) [4].

The "expanded porphyrins" are a new class of photosensitizers that have begun to attract attention [6]. Among them, the family of metallotexaphyrins has been shown to be promising because they exhibit strong absorption in the 730–770-nm spectral range, form long-lived triplet states in high quantum yield, and act as efficient photosensitizers for the production of singlet oxygen [7]. Unfortunately, most of them have relatively poor solubility in aqueous solution, and some have short stability in phosphate buffered saline or tissue culture medium. An exception is the Lu(III) derivative PCI-0123 (lutetium-texaphyrin) [8]. It has both good water solubility and apparent stability in solution. It has an absorption peak in aqueous solution centered at 733 nm, which allows for deeper penetration of the activating light than do many other photosensitizers being investigated.

Compared with other photosensitizers [1,4], few studies have been done on the PDT potential of the "expanded porphyrins." Initial work has been done in cellular [9,10], bacterial [11], and viral systems [12]. More recently, studies have begun to appear in which the photodynamic activity of texaphyrins in mammalian tumor models has been examined [8,13–16], and limited clinical trials have been done on metastatic cancers [17]. These studies, however, have not fully examined the effects of the different treatment parameters on the efficacy of PCI-0123, and only one [13] directly compared a texaphyrin compound with another photosensitizer. The present study explores the PDT response of the SMT-F tumor model to treatment with PCI-0123 as a function of laser energy dose, drug dose, and timing of laser irradiation after drug administration. It also compares the PDT efficacy of PCI-0123 with that of Photofrin®.

MATERIALS AND METHODS

Photosensitizers

A stock solution of PC-0123 (Pharmacyclics, Sunnyvale, CA) at a concentration of 2 mg/ml was prepared in 5% mannitol solution and filter sterilized. Photofrin® (Quadra Logic Technologies, Inc., Vancouver, BC, Canada) was prepared according to the manufacturer's directions by using sterile 5% dextrose to produce a final concentration of 2.5 mg/ml. The solutions were used within 6 hr of preparation.

Laser Systems

For PCI-0123, studies were conducted at 740 nm and 732 nm. The 740-nm laser light was supplied by an argon-pumped titanium/sapphire ring laser (Model 899, Coherent, Palo Alto, CA). Studies done at 732 nm were conducted by using a Lambda Plus argon-pumped dye laser (Coherent). For both systems, the beam was directed through a 400-μm fiber (PDT Systems, Santa Barbara, CA) with a microlens tip, and the output was adjusted to 150 mW/cm^2 at the tumor surface. For the Photofrin® experiments, an Innova 100 argon laser (Coherent) with a Coherent 599 dye laser tuned to 630 nm was used, with the output adjusted to 150 mW/cm^2 at the tumor surface. Power output for all lasers was determined using a Coherent 210 power meter. The beam field was expanded to a 1.5-cm-diameter spot to cover the entire tumor.

Animal Tumor Model

Female DBA/2 mice (Jackson Laboratories, Bar Harbor, ME), 6–8 weeks of age, were injected subcutaneously on the flank with a cell suspension of approximately 5×10^5 SMT-F tumor cells [18] in a volume of 0.05 ml. Cells were prepared by excising tumors from donor mice, pressing the tumor through a 40-mesh sieve (Sigma, St. Louis, MO), washing the cell suspension in cold serum-free RPMI 1640 (Life Technologies, Grand Island, NY) with penicillin (100 IU/ml) and streptomycin (100 μg/ml), pelleting the cells for 8 min at 200_{xg} relative centrifugal field in a laboratory centrifuge, and resuspending the cells at a concentration of 10^7 cells/ml in fresh medium. Where many animals were to be injected, tumors from multiple donors were pooled after sieving the tumors.

PCI-0123 Spectral Analysis

Absorption spectra for PCI-0123 were determined in both the presence and absence of sieved

tumor pieces. The spectra were generated with a DU/7 spectrophotometer (Beckman Instruments, Fullerton, CA) with the capability of background subtraction. All samples were scanned between 300 and 800 nm. Cell-free PCI-0123 was prepared by diluting the 2 mg/ml stock PCI-0123 solution with RPMI + 10% fetal bovine serum (FBS) to a concentration of 50 μg/ml. RPMI + 10% FBS was used for the background subtraction. Tumor-bound PCI-0123 was prepared by adding sieved tumor pieces containing approximately 10^6 tumor cells for each milliliter of 50 μg/ml PCI-0123, incubating the suspension for 6 hr in a 5% CO_2 incubator, and then washing the suspension twice with phosphate buffered saline (PBS) to remove excess, i.e., unbound, PCI-0123 prior to scanning in RPMI. The pH effects were tested for by buffering the incubation solution at pH 6.5, 7.4, or 8.5. A matched, nonphotosensitizer-exposed suspension of sieved, incubated, washed tumor pieces in RPMI was used for background subtraction. An additional test was done where the cells were incubated in 100% serum (pH 7.4), washed with PBS, and measured against a matched unexposed control.

PDT Treatment

When the tumors reached 4–7 mm in diameter, the animals were placed into one of the following treatment groups: (a) photosensitizer alone (no light), (b) solvent alone (no light), (c) laser light alone, (d) different combinations of light and photosensitizer doses. Both the photosensitizers, PCI-0123 or Photofrin®, and their solvents, 5% mannitol or 5% dextrose, were injected through the tail vein. During the laser exposure, the animals were anesthetized with a ketamine (50 mg/kg) and xylazine (10 mg/kg) cocktail given intraperitoneally to prevent movement of the animal in the beam field. All light-exposed tumors were treated with 150 J/cm^2 of cw laser light delivered at a power density of 150 mW/cm^2. Each experimental group consisted of three to eight animals chosen at random from the tumor-bearing population. The maximum post-PDT observation period was 45 days for animals showing complete cure. Each experiment was done at least twice.

Response of the tumors to PDT treatment was divided into four categories:

No response: the tumor continued to grow normally with no noticeable change

Partial response: the tumor growth was temporarily halted or the tumor mass was partly reduced, but the tumor resumed normal growth during the observation period

Full response: the original tumor mass was reduced to a visually undetectable/impalpable level, but the tumor regrew during the observation period

Cure: the tumor mass was reduced to an undetectable level during the entire observation period.

Tumor Size Measurements

Tumor size was monitored for each tumor by measuring two diameters perpendicular to each other with a digital readout caliper (Fowler, Ultracal II, Sylvac, Switzerland). The volume of the tumor was estimated from the formula for a prolate ellipsoid with short equal axes and π approximated as 3, i.e., $V = \frac{1}{2}L \times W^2$ [19]. Measurements were made at the time of irradiation and three times per week after treatment until the animal was removed from the study. Animals were removed from the study when their tumors either exceeded 1,700 mm^3 or impaired the animal's movement. To allow for comparison from experiment to experiment, changes in tumor size were normalized by dividing each the new volume of each tumore by its starting volume at the time of treatment.

RESULTS

Spectrum of PCI-0123 in Tumor Cells

The spectrum of tumor pieces exposed for 6 hr to 50 μg/ml of PCI-0123 in RPMI + 10% FBS was compared with that of PCI-0123 alone in RPMI + 10% FBS.

Figure 1A shows that PCI-0123 in medium + 10% FBS at physiologic pH 7.4 has three main absorption peaks at 417, 470, and 733 nm. The ratio of the absorption peak at 417 nm to the peak at 470 nm is approximately 0.84:1. The 733-nm peak to 470-nm peak ratio is 0.63:1. The 733-nm peak falls off at a rate of 0.03 absorption units for 10 nm of wavelength shift.

Figure 1B demonstrates three changes that occur when tumor pieces (consisting of tumor cells, vessel fragments, and connective tissue) are incubated with the photosensitizer and the medium at pH 7.4, which is considered to be physiologic conditions, or in 100% serum (not shown, but the same as the pH 7.4 curve). First, there is a slight shift of the both second and third peaks of

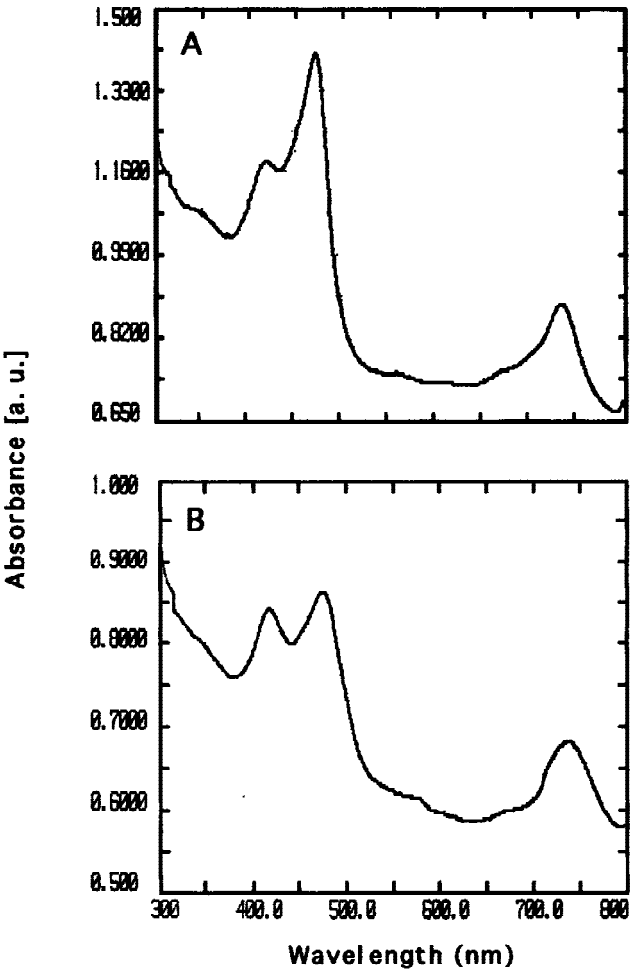

Fig. 1. Absorption spectra for cell-free PCI-0123 and sieved tumor-bound PCI-0123. **A:** Absorption spectrum of 50 μg/ml PCI-0123 in RPMI + 10% fetal bovine serum (FBS), pH 7.4. **B:** Absorption spectrum of PCI-0123 bound to sieved tumor "chunks" after 6 hr of incubation with 50 μg/ml PCI-0123 in RPMI + 10% FBS, pH 7.4.

3 nm toward the red. Second, there is definite a broadening of the third, longer wavelength, peak. This peak, now centered at 736 nm rather than at 733 nm, is essentially flat (absorption difference < 0.003) between 729 and 742 nm. Third, there is a change in the ratios of the different absorption peaks. The 417:473-nm ratio is now only 0.97:1 and the 736:473-nm ratio is 0.78:1.

Figure 2 shows the portion of the spectrum containing the third peak, when the cells were incubated in uptake conditions of either pH 6.5 or 7.4. At pH 7.4 the peak is at 736 nm. The absorption maximum is shifted to 730 nm, and the peak is narrowed at pH 6.5. When incubated under basic conditions (pH 8.5), a further red shift was seen, producing a peak at 738 nm (data not shown). The shorter wavelength portion of the spectrum was the same for each of the pH conditions tested.

Toxicity Determination for PCI-0123

Animals were injected through the tail vein with 1, 2.5, 5, 10, 20, 40, or 100 mg/kg PCI-0123 in 5% mannitol and monitored for 72 hr for adverse effects. Below 40 mg/kg, no effects were seen. At 100 mg/kg, the mice suffered seizures and died within 5 min of injection. With 40 mg/kg, some mice (<16 g) exhibited an initial acceleration of heart rate, rapid breathing, and tremors. These reactions subsided within 5–10 min, after which the mice appeared normal in behavior. By selecting larger (>18 g), i.e., slightly older mice, this reaction was avoided. All mice excreted colored urine, increasing in darkness with increasing drug dose, beginning approximately 1 hr after drug injection. The color reached its deepest about 6–8 hr after injection and returned to normal by 24 hr after injection.

Effect of Increasing Drug Dose

Mice were given 1, 2.5, 10, or 40 mg/kg of PCI-0123 IV. Five hours later, they were exposed to 150 J/cm^2 of 740 nm laser light at a dose rate of 150 mW/cm^2. As seen in Figure 3, increasing doses of PCI-0123 produced increasingly better tumor reduction, with 40 mg/kg (the maximum nontoxic dose) producing the best response.

Effect of Changing the Laser Light Dose

Tumor response to laser light doses of 150, 300, 450, and 600 J/cm^2 was determined in mice given 10 mg/kg PCI-0123 and exposed to 732 nm laser light 5 hr later. As seen in Figure 4, there was no significant difference in the growth of the tumors when exposed to increasing light doses. Nearly all (86%) of the PDT mice developed ulcers in the irradiation site. There was no correlation with ulceration and light dose. In the control mice given 300 and 450 J/cm^2, a reddening and some edema was seen in the irradiated area. Control mice exposed to 600 J/cm^2 had not only more pronounced reddening and swelling but also experienced a drying/flaking of the exposed area, and one mouse developed a small blister in the irradiated spot. Only the mice exposed to 150 J/cm^2 had no skin irritation or edema.

Selection of Postinjection Irradiation Time

Animals were given 40 mg/kg PCI-0123 IV and then exposed to 150 J/cm^2 of 740 nm laser light at 1, 3, 5–6, 12–14, or 24 hr after injection. All animals treated at 1 hr postinjection died

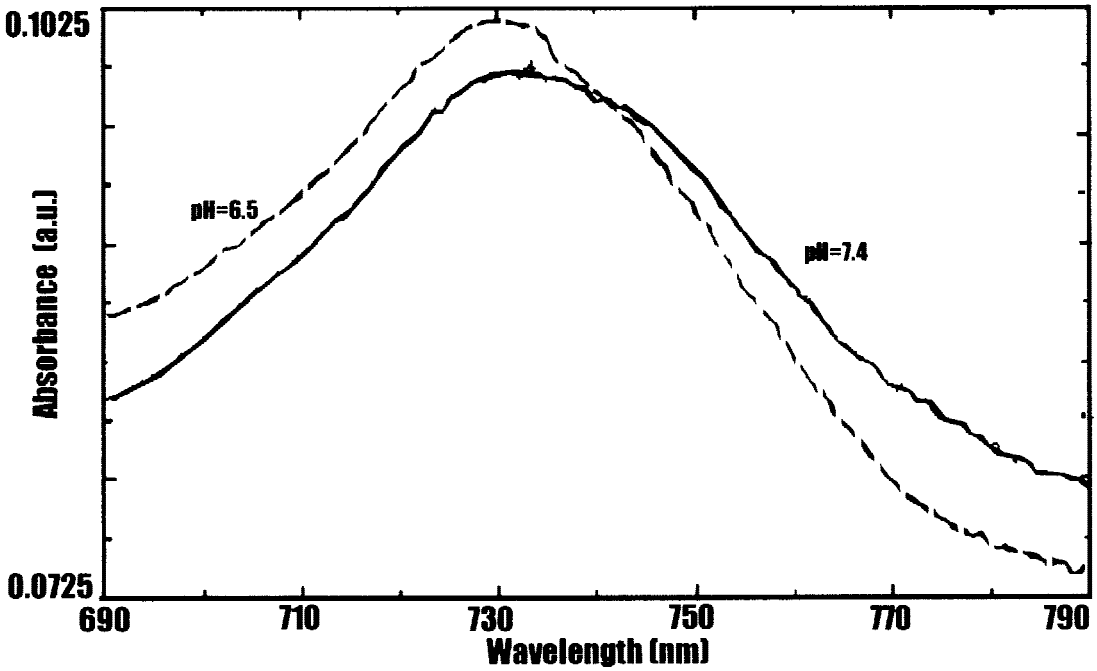

Fig. 2. Portion of the absorption spectrum containing the third absorption peak, showing the effect of acidic pH (6.5) versus physiologic pH (7.4) on the absorption peak position and breadth. Tumor "chunks" were incubated for 6 hr with 50 μg/ml PCI-0123 in RPMI + 10% fetal bovine serum at pH 6.5 (broken line) or 7.4 (solid line).

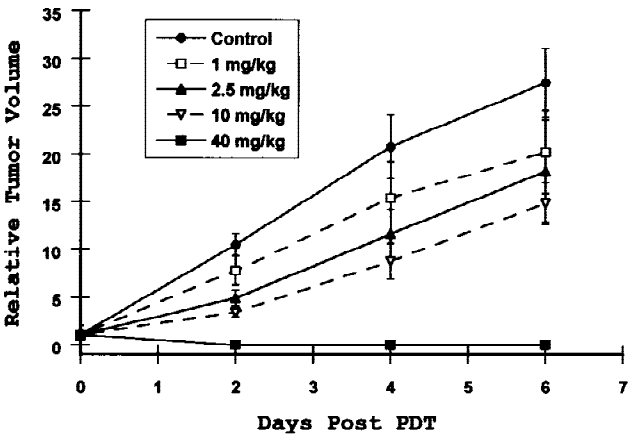

Fig. 3. Typical tumor response to photodynamic therapy (PDT) treatment as a function of the concentration (mg/kg body weight) of PCI-0123 given intravenously 6 hr before irradiation with 150 J/cm^2 light at 740 nm. Tumor volume changes are relative to the initial tumor volume at the time of PDT treatment. The control group (n = 6) is a combination of mice receiving either light alone (n = 3) or drug (40 mg/kg) alone (n = 3) because the treatments were indistinguishable ($P < 0.05$). Other groups contained either three mice (1, 2.5, 40 mg/kg) or four mice (10 mg/kg). Data points are the mean of each group. Error bars = SEM.

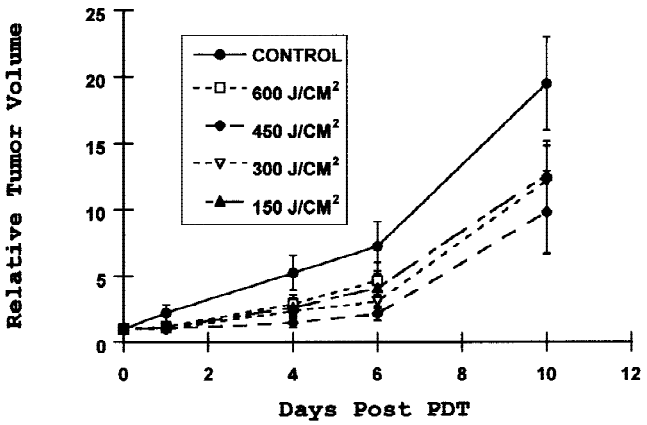

Fig. 4. Typical tumor response to photodynamic therapy (PDT) treatment as a function of changing the total 732-nm light dose given (J/cm^2) 6 hr after intravenous administration of 10 mg/kg PCI-0123. Tumor volume changes are relative to the initial tumor volume at the time of PDT treatment. Controls (n = 4) received 600 J/cm^2 of 732 nm light alone. Other groups contained either three mice (450 and 600 J/CM^2) or four mice (150 and 300 J/CM^2). Data points are the mean of each group. Error bars = SEM.

within 24 hr following the treatment. Animals treated 3 hr postinjection exhibited extensive tissue damage around the tumor site, including sloughing of the normal skin area, had deep ulceration of the tumor area, and showed greatly impaired hindlimb movement. The tumor tissue did not respond evenly to the treatment, with portions of the tumor remaining next to areas of erosion. None of the wounds healed well. During the first two days posttreatment, most of the animals

TABLE 1. Photodynamic Therapy Treatment Time Determination

Group	n	Tumor response			Mean (±SEM) survival (days)	Maximum survival (days)
		None	Part/full	Cure		
Control	20	20			5 ± 1	6
3 hr after drug	7	2	4/1		6.5 ± 4	14
6 hr after drug	8		2/2	4	31 ± 12	45
12 hr after drug	7	1	2/4		13.5 ± 3	17
24 hr after drug	5	3	2/0		7 ± 3	9

developed a general body swelling. Many of the animals had to be euthanized within seven days of treatment. The group treated 5–6 hr postinjection showed the best response. They had less overall tissue erosion than did the 3-hr group but did slough the entire tumor area 48–96 hr after PDT. They had some initial general edema during the first 24–36 hr after treatment but less than the 3-hr group. They also exhibited an initial impairment of hindlimb movement but to a lesser degree than did the 3-hr group. Their mobility improved with time until the survivors were normal by the end of the observation period. Depending on the experiment, 50–67% of the mice remained tumor free during the entire posttreatment observation period. The 12–14-hr postinjection group showed significantly less response to the PDT treatment than did the 5–6-hr group. Although there was some tissue reaction, the loss was mostly from the top of the tumor. After a period of stasis, the tumors regrew at rates near those of the controls. Most animals treated 24 hr postinjection showed no response. The partly responding tumors showed only a slowed growth rate with no tissue loss. Table 1 presents the typical PDT response of tumors as a function of the different postinjection treatment times. Clearly, the best PDT effects and the longest survival times were achieved in the 5–6-hr postinjection group.

Phototoxicity Comparison with Photofrin®

Tumor-bearing mice were injected with either 40 mg/kg PCI-0123 or 10 mg/kg Photofrin®. PCI-0123-treated mice were irradiated with 740 nm laser light 5–6 hr after drug injection. Photofrin®-treated mice were irradiated 24 hr after injection with 630 nm laser light. All mice were treated with 150 J/cm^2 delivered at 150 mW/cm^2. The PCI-0123 group had substantial tissue slough within 48 hr and some movement impairment but healed well and improved with time. Preceding the tissue slough, all of the animals developed a darkened area (hemorrhage?) under the skin. All of the mice responded to the treatment, and 40% of the mice remained tumor free for the entire posttreatment observation period. The Photofrin® group did not exhibit substantial tissue slough, although there was marked shrinkage of the tumor in 67% of the mice. Sixteen percent of the Photofrin® mice exhibited a complete cure. As evident from the graphs in Figure 5, the PDT response of the tumors treated with PCI-0123 was greater and longer lasting than that seen with Photofrin® treatment.

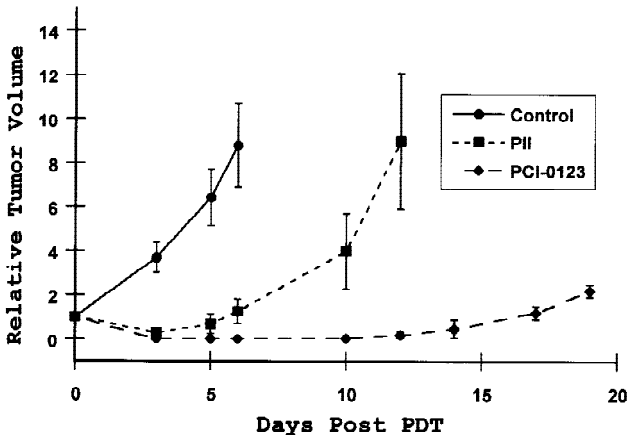

Fig. 5. Comparison of the photodynamic therapy (PDT) response of tumors treated with Photofrin® (10 mg/kg) and 150 J/cm^2 laser light at 630 nm with tumors treated with PCI-0123 (40 mg/kg) and 150 J/cm^2 laser light at 740 nm. Tumor volume changes are relative to the initial tumor volume at the time of PDT treatment. The control group (n = 16) is a composite of mice receiving either light alone (n = 4, 630 nm; n = 4, 740 nm) or photosensitizer alone (Photofrin®, n = 4; PCI-0123, n = 4) because the groups were indistinguishable ($P < 0.05$). For the Photofrin®-treated group, n = 6. For the PCI-0123-treated group, n = 8. Data points are the mean of each group. Error bars = SEM.

DISCUSSION

The spectral changes seen when PCI-0123 was incubated with tumor material under the most physiologic conditions, pH 7.4 and/or 100% serum, are consistent with those seen in other porphyrins as they "age" [20,21]. Changes in the relative intensity of the absorption peaks could be

due to the PCI-0123 being metabolized by the cells, aggregation of the drug in the more hydrophobic environment of the cell interior, and/or differences in pH in the interior of the cell versus that of the cell-free solution. The slight red shift in the peaks is most likely due to the nature of the porphyrin-binding protein in the cells being different than that in the cell-free medium [22,23]. Kostenich et al. [15] found, when using laser-induced fluorescence to obtain spectra after administration of PCI-0123, that the peak not only became red shifted but also that it continued to shift for up to 24 hr after drug administration, indicating that there were ongoing changes in the microenvironment of the dye or possibly biomodification of the dye by the tissue. A similar shift in fluorescence spectra was seen by Woodburn et al. [14] who found that the dye was being accumulated in the lysosomes of the EMT-6 cells. However, we have not been able to show analogous localization of the dye in the SMT-F tumor cells. The shifting and broadening of the longest wavelength absorption peak when incorporated into the tumor cells is advantageous because it allows for a wider range of wavelengths that can be used to potentially give deeper penetration of the absorbed light.

We examined the effect of increasing the PCI-0123 dose on the PDT response of the tumors while using a light dose comparable to that used for PDT with other photosensitizers [24,25]. Only exposure to 40 mg/kg of PCI-0123 consistently resulted in a response of all treated tumors. Because 40 mg/kg PCI-0123 in combination with 150 J/cm^2 of 740 nm laser light was so effective, we chose a lower concentration of drug (10 mg/kg) to examine whether the same efficacy could be achieved by increasing the light dose at the peak of the free drug absorption (732 nm) while decreasing the amount of drug needed. It was found that no substantial increase in PDT response was obtained by increasing the total light dose. The most likely explanation for this finding is that all the available compound had reacted with the light even at the lowest total joules used in this study. Work by Kostenich et al. [15] and Woodburn et al. [16] supports this possibility. Both groups of investigators found that, at drug concentrations comparable to ours, increasing the fluence above 150 J/cm^2 did not increase efficacy. In addition, Kostenich et al. found that at lower fluences there was a light–dose-dependent tumor response. Furthermore, Woodburn et al. found that, by increasing the drug dose twofold, better average survival was achieved but there was still no light–dose-dependent response. All the available compound was probably reacted with the lowest fluence light used. Therefore, it was concluded that the light application timing and Photofrin® comparison experiments should be done with the highest light dose, which showed no evidence of damage to normal tissues (150 J/cm^2) in combination with the highest drug dose that was well tolerated (40 mg/kg).

This study, like those of Young et al. [8], Konig et al. [13], and Woodburn et al. [14,16], found that early light application times, 5–6 hr after drug injection, were most effective. The diminished PDT response at 24 hr after drug administration and the observation that the urine color had returned to normal by that time indicates that most of the unbound photosensitizer had cleared from the animals by that time. At the earliest irradiation time tested by Young et al. [8] or Woodburn et al. [14,16], 3 hr postinjection, we found much greater general damage than they did. Because we used a higher concentration of PCI-0123, this result may be due to higher circulating levels of PCI-0123 that have not yet had time to develop a differential tissue localization. The deaths at the earliest irradiation time, 1 hr postinjection, may be due to either toxic products formed from the irradiation of a large amount of free drug in the blood or traumatic shock syndrome [26], which would also be related to a high drug concentration in the blood.

PCI-0123 appears to be a more effective photosensitizer than Photofrin®. With Photofrin® treatment, only 67% of the tumors responded, whereas 100% of the PCI-0123 tumors showed response. In addition, more than twice as many PCI-0123-treated mice (40%) remained tumor free during the observation period, as did Photofrin®-treated mice (16%), which may be due in part to greater tissue penetration of the light at 740 nm versus that at 630 nm used for Photofrin® [27,28]. It also could be the result of more efficient production of singlet oxygen by PCI-0123 [7,29], which would lead to greater cytotoxicity at the drug localization site. Our finding of greater efficacy with PCI-0123 than with Photofrin® is opposite to that found by Konig et al. [13] when comparing Cd-texaphyrin with hematoporphyrin derivative. The most likely explanation of this difference is that the metal species in the center of the texaphyrin moiety affects the usefulness of that particular texaphyrin compound as a photosensitizer.

Although we did not measure the amount of either PCI-0123 or Photofrin® in our tumors, it is possible from the literature to estimate the amounts present. Bellnier et al. [30] found that after 24 hr mice with SMT-F tumors given 5 mg/kg photofrin II (the nonclinical predecessor of Photofrin®) retained 2–5 μg of photosensitizer per gram of tumor. Their work showed a linear uptake over the dose range of 5–27 mg/kg. Therefore, it is reasonable to assume that our mice retained 4–20 μg of Photofrin® per gram of tumor. In the work of Young et al. [8], SMT-F tumor-bearing mice were found to have retained 4.72 μg of PCI-0123 per gram of tumor when given 10 mg/kg of photosensitizer. Assuming linearity, it would be reasonable to predict that our mice retained about 19 μg of PCI-0123 per gram of tumor. Given that only two to three mice were used to determine each of the values reported in these studies [8,30], it seems reasonable to state that the amounts of the photosensitizers retained by the tumors in our study are essentially equal. Differences in efficacy are probably not due to differences in compound retention.

An examination of the extinction coefficients for the two photosensitizers also fails to explain the efficacy difference. Kostenich et al. [15] found that PCI-0123 had a coefficient of 18,875 in water, whereas Kimel et al. [31] used a value of 4,400 for Photofrin®-loaded tissue culture cells in their study of singlet oxygen generation. This fourfold difference would seem to favor Photofrin® as the more efficient photosensitizer, but efficiency is not the only determinant of efficacy. Other factors such as depth of light penetration, site of photosensitizer localization within the tissue(s) and cells, and photobleaching of the photosensitizer also influence the final treatment outcome.

The differences in the amount of PCI-0123 used in the present study and that used by others may be explained by a number of factors. Young et al. [8] found that larger tumors required higher concentrations of drug be administered. The tumors in our study were closer to the size of their larger neoplasm group, which received half the amount of our mice but also had a lower cure rate. The tumors in the study by Woodburn et al. [14] were of a different type, EMT-6, which is immunogenic and generally more sensitive to PDT treatment than are other tumor types. In fact, Margaron et al. [32] reported that the EMT-6 tumor responds fully to Photofrin®-mediated PDT at doses well below those used in our study, where the response was not complete. Thus, the use of less PCI-0123 by Woodburn et al. is not surprising. The study by Kostenich et at. [15] is not really comparable for amount used because not only did they use a different tumor, they also delivered the drug by a different route, used a different irradiation time, and did not use a single wavelength light source.

The PDT response seen in the PCI-0123 mice was greater but less localized than that seen with Photofrin®. In all instances when a PDT response was seen with PCI-0123, there was a darkening of the irradiated area that was not seen in the mice treated with Photofrin®. Preliminary work on the mechanism of the effects of PCI-0123 indicates that the vasculature may be one of the primary targets (Liaw, unpublished observations). If that is the case, then any subcutaneous vessels/capillaries exposed to the laser light could hemorrhage, thus explaining the darkening under the skin of the irradiated area. None of the Photofrin® mice had tissue slough that resulted in open wounds, although several of the PCI-0123 mice did, especially those treated at the earlier postinjection irradiation times. In addition, none of the Photofrin® mice exhibited any impairment in their mobility as was observed with the PCI-0123 mice. Both of these differences could be explained by a more uniform tissue distribution of PCI-0123 than of Photofrin®, which could be due to either the short time the PCI-0123 remains in the animal's system or the high concentration of PCI-0123 used, which may result in a high free drug concentration in the plasma. Certainly, an even distribution of the photosensitizer would account for the tissue slough seen with PCI-0123 because all the tissues exposed (skin, tumor, muscle) would be expected to undergo similar degrees of tissue damage at similar depths. Also, the impaired hindlimb movement could be explained if underlying muscle tissue is damaged as a result of retained drug that reacts to the deeper penetrating light.

These studies demonstrate a strong PDT effect of PCI-0123. An understanding of the mechanism(s) of its action will allow better evaluation of its potential as a clinically useful photodynamic agent. Further studies are underway to identify the subcellular, cellular, and tumor target sites of the PDT response in this system.

ACKNOWLEDGMENTS

We thank Jeff Andrews for assistance with the laser systems, Dr. Chung-Ho Sun for assistance with the spectral analysis, Glen Profeta for

assistance with figure preparation, and Pharmacyclics for the gift of lutetium-texaphyrin (PCI-0123).

REFERENCES

1. Henderson BW, Dougherty TJ. How does photodynamic therapy work? Photochem Photobiol 1992;55:145–157.
2. Dougherty TJ. Photosensitizers: therapy and detection of malignant tumors. Photochem Photobiol 1987;45:879–889.
3. Gomer CJ, Rucker N, Ferrario A, Wong S. Properties and applications of photodynamic therapy. Radiat Res 1989; 120:1–18.
4. Fisher AMR, Murphree AL, Gomer CJ. Clinical and preclinical photodynamic therapy. Lasers Surg Med 1995; 17:2–31.
5. Foote CS. Chemical mechanisms of photodynamic action. Proc SPIE 1990;6:115–126.
6. Sessler JL, Murai T, Lynch V, Cyr M. An "expanded porphyrin": the synthesis and structure of a new aromatic pentadentate ligand. J Am Chem Soc 1988;110:5586–5588.
7. Harriman A, Maiya BG, Murai T, Hemmi G, Sessler JL, Mallouk TE. Metallotexaphyrin: a new family of photosensitizers for efficient generation of singlet oxygen. J Chem Soc Chem Commun 1989:314–316.
8. Young SW, Woodburn KW, Wright M, Mody TD, Fan Q, Sessler JL, Dow WC, Miller RA. Lutetium texaphyrin (PCI-0123): a near-infrared, water soluble photosensitizer. Photochem Photobiol 1996;63:892–897.
9. Ehrenberg B, Malik Z, Nitzan Y, Ladan H, Johnson FM, Hemmi G, Sessler, JL. Photosensitization of bacterial and animal cells with the new photosensitizer benzoporphyrin and texaphyrin [abstract A43]. Fourth Congress of the European Society for Photobiology. Amsterdam, September 1991.
10. Sessler JL, Hemmi G, Mayia BG, Harriman A, Judy ML, Boriak R, Matthews JL, Ehrenberg B, Malik Z, Nitzan Y, Ruck A. Tripyrroledimethine-derived ("texaphyrin"-type) macrocycles: potential photosensitizers which absorb in the far-red spectral region. Proceedings SPIE Intl Opt Eng 1991;1426:318–329.
11. Ehrenberg B, Malik Z, Nitzan Y, Ladan H, Johnson FM, Hemmi G, Sessler JL. The binding and photosensitization effects of tetrabenzoporphyrins and texaphyrin in bacterial cells. Lasers Med Sci 1993;8:197–203.
12. Matthews JL, Sogandares-Bernal F, Judy M, Gulliya K, Newman J, Chanh T, Marengo-Rowe A. Inactivation of viruses with photoactive compounds. Blood Cells 1992; 18:75–88.
13. Konig K, Genze F, Miller K, Ruck A, Reich E, Repassy D. Photodynamic activity of liposome-delivered Cd-texaphyrin using tumor-bearing nude mice. Lasers Surg Med 1993;13:522–527.
14. Woodburn KW, Fan Q, Miles DR, Kessel D, Lou Y, Young SW. Localization and efficacy analysis of the phototherapeutic lutetium texaphyrin (PCI-0123) in the murine EMT6 sarcoma model. Photochem Photobiol 1997;65: 410–415.
15. Kostenich G, Orenstein A, Roitman L, Malik Z, Ehrenberg B. In vivo photodynamic therapy with the new near-IR absorbing water soluble photosenistizer lutetium texaphyrin and a high intensity pulsed light delivery system. J Photochem Photobiol B Biol 1997;39:36–42.
16. Woodburn KW, Fan Q, Kessel D, Luo Y, Young SW. Photodynamic therapy of B16F10 murine melanoma with lutetium texaphyrin. J Invest Dermatol 1998;110:746–751.
17. Renschler MF, Yuen A, Panella TJ, Wieman TJ, Julius C, Panjehpour M, Taber S, Fingar V, Horning S, Miller RA, Lowe E, Engel J, Woodburn K, Young SW. Photodynamic therapy trials with lutetium texaphyrin (Lu-Tex). Photochem Photobiol 1997;65:47S.
18. Pavelic ZP, Porter CW, Allen LM, Mihich E. Cell population kinetics of fast- and slow-growing transplantable tumors derived from spontaneous mammary tumors of the DBA/2 Ha-DD mouse. Cancer Res 1978;38:1533–1538.
19. Simpson-Herren L, Lloyd HH. Kinetic parameters and growth curves for experimental tumor systems. Cancer Chemother Rept 1976;54:143–174.
20. Bottiroli G, Freitas I, Docchio F, Ramponi R, Sacchi CA. The time-dependent behavior of hematoporphyrin-derviative in saline: a study of spectral modifications. Chemico-Biol Interact 1984;49:1–11.
21. Kalia I, Jain V. Effects of some divalent metal ions on the aging phenomenon of hematoporphyrin and Photofrin®. Indian J Biochem Biophys 1989;26:213–218.
22. Smith A, Neuschatz T. Haematoporphyrin and OO′-diacetylhaematoporphyrin binding by serum and cellular proteins. Implications for the clearance of these photochemotherapeutic agents by cells. Biochem J 1983;214: 503–509.
23. Bottiroli G, Docchio F, Freitas I, Ramponi R, Sacchi CA. Spectroscopic studies of hematoporphyrin-derivative in culture medium. Chemico-Biol Interact 1984;50:153–157.
24. Evensen JF, Moan J. A test of different photosensitizers for photodynamic treatment of cancer in a murine tumor model. Photochem Photobiol 1987;46:859–865.
25. Richter AM, Waterfield E, Jain AK, Canaan AJ, Allison BA, Levy JG. Liposomal delivery of a photosensitizer, benzoporphytin derivative monoacid ring A (BPD), to tumor tissue in a mouse tumor model. Photochem Photobiol 1993;57:1000–1006.
26. Ferrario A, Gomer CJ. Systemic toxicity in mice induced by localized porphyrin photodynamic therapy. Cancer Res 1990;50:539–543.
27. Svaasand LO. Optical dosimetry for direct and interstitial photoradiation of malignant tumors. In: Doiron DR, Gomer CJ, editors. Porphyrin localization and treatment of tumors. New York: Alan R. Liss, Inc. 1984; p 91–114.
28. Wilson BC. Photodynamic therapy: light delivery and dosage for second-generation photosensitizers. In: Photosensitizing compounds: their chemistry, biology and clinical use. Ciba Foundation Symposium 1989;146:61–73.
29. Redmond RW, Heihoff K, Braslavsky SE, Truscott TG. Thermal-lensing and phosphorescence studies of the quantum yield and lifetime of singlet molecular oxygen (1 delta g) sensitized by hematoporphyrin and related porphyrins in deuterated ad non-deuterated ethanols. Photochem Photobiol 1987;45:209–213.
30. Bellnier DA, Ho Y-K, Pandey RK, Missert JR, Dougherty TJ. Distribution and elimination of Photofrin II in mice. Photochem Photobiol 1989;50:221–228.
31. Kimel S, Tromberg BJ, Roberts WG, Berns MW. Singlet oxygen generation of porphyrins, chlorins and phthalocyanines. Photochem Photobiol 1989;50:175–183.
32. Margaron P, Madarnas P, Quellet R, Van Lier JE. Biological activities of phthalocyanines. XVII. Histopathologic evidence for different mechanisms of emt-6 tumor necrosis induced by photodynamic therapy with disulfonated aluminum phthalocyanine or Photofrin. Anticancer Res 1996;16:613–620.